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14. ABSTRACT

The incidence and mortality of prostate cancer (PCa) is approximately 2 fold higher in African American (AA) than in European-American (EA) men. This disparity is believed to be a complex combination of environment, socioeconomic factors and genetics. The purpose of the present study is to study genome wide DNA methylation changes in prostate biospecimens from AA and EA men in order to elucidating the epigenetic DNA methylation changes associated with prostate cancer disparity and identify novel biomarkers for early disease detection**.** **RESULTS**: In our preliminary genome-wide methylation studies, we examined the methylation status of Infunium 450K (484,968) CpG sites that corresponds to 21,221 genes in microarray (illumina) analysis. In all, we selected 25 promoter associated novel CpG sites that were differentially methylated in correlation with prostate cancer progression from benign to HGPIN to prostate cancer (FDR adjusted p-value <0.05; b value >0.2; fold change > 1.5). Several novel genes demonstrated significant difference in methylation patterns in AA versus EA prostate cancer cell lines. **Conclusion:** Our on-going genome-wide methylation approach based on the methyl-binding domain of MBD2 (qMBD-seq) coupled with newly developed computational methods has the advantage that we can now obtain genome-wide methylation data without bias to specific regions in comparison with other independent methodologies such as the Infunium 450K array and this will improve the quantitative determination of DNA methylation status across sample groups (e.g. AA versus EA samples). Concerted efforts to accurately study genome wide DNA methylation changes in prostate biospecimens from AA in comparison to EA men is needed in order to fully understand the molecular mechanisms underlying PCa disparity if genome knowledge-based perspective can be used to eliminate PCa health disparity.

15. SUBJECT TERMS
GENOME-WIDE DNA METHYLATION CHANGES, PYROSEQUENCING, RT-PCR, WESTERN BLOT ANALYSIS, PROSTATE CANCER, AFRICAN AMERICAN , EUROPEAN-AMERICAN MEN

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INTRODUCTION

Prostate cancer (PCa) is a common malignancy and a leading cause of cancer death among men in the United States. Racial differences in PCa incidence and mortality are well documented. The incidence and mortality for PCa is about twofold higher in African-American (AA) in comparison with European-American (EA) men, with AA men experiencing among the highest rates worldwide[1]. African-American men have a 60% increased risk of developing prostate cancer, twice the risk of developing distant disease and twice the mortality relative to their European-American counterpart [2]. The disparity in PCa is believed to be a complex combination of socioeconomic factors, environment and genetics [3]. Genetic differences including mutation, loss or the amplification of different chromosomal regions may also play a major role in the disparity observed in prostate cancer incidence and mortality between AA and EA men. Recent advances in genomic studies have identified several genes involved in pathways relevant to prostate cancer biology; in particular, a number of genes with alleles which differ in frequency between AA and EA have been proposed as a genetic cause or contributor to the increased risk of PCa in AA. Genes that are involved in the sex steroid hormones and components of their signaling and metabolic pathways has been extensively studied in relation to prostate cancer susceptibility and shown to differ in AA and EA populations [4]. To date, single-nucleotide polymorphisms (SNPs) identified on 8q24 is well documented as risk alleles for AA men with prostate cancer [5]. However, the risk variants are located in non-protein coding regions and the biological mechanisms underlying this association remain unclear.

In addition to the genomic defects associated with increased risk of prostate cancer, epigenetic defects such as DNA methylation changes and histone modifications are increasingly found to be associated with prostate disease prevalence. There is growing evidence to suggest that epigenetic DNA methylation changes affects gene expression in an age-dependent and tissue specific manner [6]. We have recently demonstrated that indeed CpG hypermethylation increases with age in the normal human prostate tissues [7]. Such age-dependent DNA methylation changes can alter cell physiology and possibly, predispose prostate cells to neoplastic transformation. Also in many cases, aberrant methylation precedes full-blown malignancy and can often be found in non-cancerous tissues; in the prostate, hypermethylation of the GSTP1 CpG has been detected in PIA lesions [8]. DNA methylation occurs at CpG sites in the human genome and involves the addition of a methyl group to the 5'-carbon of cytosine, forming 5-methylcytosine (5-meC; [9, 10]). Since the recognition that the GSTP1 CpG island was frequently hypermethylated in PCa, more than 40 genes have been reported to be targets of DNA hypermethylation-associated epigenetic gene silencing in PCa cells [11]. Ethnic group-related differences in CpG hypermethylation has also been identified for several genes. One study demonstrated that GSTP1 hypermethylation was significantly higher in PCa samples from AA men in comparison with EA and Asians [12]. Another study found higher frequency of hypermethylation of cell adhesion molecule (CD44) hypermethylation in prostate cancer tissues from AA in comparison to EA [13] suggesting that inactivation by CpG methylation may play a role in the disparity associated with prostate carcinogenesis. Our preliminary data demonstrate higher methylation of several genes in prostate tissue samples from AA in comparison with their

EA counterparts [14]. The current literature suggests a complex mechanism of epigenetic regulation in prostate cancer including DNA methylation changes that can lead to either gene silencing or the activation of key regulatory genes in the disease pathway. Underlying this aberrant DNA methylation is the accumulating body of data hinting that normal prostate cells may be subjected to a relentless barrage of genome-damaging stresses due to both exogenous and endogenous carcinogens, with damage accumulating over time and age. Thus aberrant epigenetic DNA methylation changes may represent the integration of environmental or lifestyle exposures and genetic predisposition to prostate cancer. Such events may differ between individuals belonging the same ethnic group or individuals belonging to different ethnic groups. Thus the elucidation of genome-wide DNA methylation changes in prostate tissues from different ethnic groups would contribute to our understanding of the molecular mechanisms underlying prostate cancer disparity and potentially lead to the identification of "ethnic sensitive" biomarkers for early disease detection. We know that there are different thresholds for AA versus EA men for PSA screening. Therefore other markers such as DNA methylated genes that can clarify such ethnic sensitive screening strategies would also be helpful. Furthermore, differential methylation changes could also lead to identification of potential novel therapeutic targets for prostate cancer treatment.

In this study we sought to investigate whether differential DNA methylation changes may represent an integration of lifestyle and genetic predisposing factors to create a more aggressive disease milieu in African American patients.

BODY

As outlined in our Statement of work, we seek to accomplish 3 main tasks during the 3 years of funding. We have made substantial progress at the end of this first year of funding period. A draft manuscript describing the "Identification of distinct DNA Methylation Signatures in the Human Prostate Cancer Tissues using Illumina Infunium 450K bead Chip Genome-Wide Methylation Array" is in preparation for submission to *Epigenetic Journal*. In addition an abstract from this work was presented at the American Association for Cancer Research 2012, Annual Meeting in Chicago, IL (a copy of the abstract is attached below).

Specific Aim 1: Evaluation of genome-wide differences in DNA methylation in prostate tissues from African- American versus European-American Men (months 0 – 24):

Task 1: DOD regulatory review and approval processes for studies involving human samples (months 0 – 6).

Regulatory review of human subjects' research has been completed, including at the DOD and at Howard University and Johns Hopkins University.

Task 2: Perfect bioinformatics tools for analysis of genome-wide methylation data (months 0 – 6).

During Year 1 of the project, we have been able to complete this critical task to perfect the computational/bioinformatics analytical methods for the qMBD-seq genome-wide DNA methylation technology. The qMBD-Seq approach features use of the methyl-

binding domain of the MBD2 protein (MBD2-MBD) to bind and enrich methylated DNA fragments with high avidity and specificity. The resulting library of methylated DNA fragment is then analyzed by next generation sequencing. Comparison with an un-enriched total input should theoretically yield estimates of the degree of methylation at any given location in the genome. However, as with any affinity enrichment based approach for analysis of DNA methylation (e.g. MeDIP), the signals for a given sample and at a given locus are not on the same scale, which is influenced by multiple parameters, including CpG density, overall degree of methylation, sequence biases. This makes quantitative determination of DNA methylation and assessment of differential methylation across sample groups (e.g. African American vs. European-American) very challenging. If each sample has a separate scale for the DNA methylation estimates, then comparative analysis with typical statistical tests can lead to a high degree of false negative and false positive results.

In year one of this project, we have developed a novel complex internal standard approach to correct for these biases and put the DNA methylation estimates on an absolute scale between 0 and 1 for all samples. This involves spiking each sample with a fully methylated complex internal standard and developing a model based approach to adjust for these confounding parameters in the signals obtained for this internal standard. We have seen that the relationship of the number of reads at a given 150 bp window (the signal) is highly linearly related to the number of methylated CpGs (see Figure below). We can then understand this relationship for each sample and adjust for this effect in order to put the methylation estimates for each sample on the same scale. With this internal spike-in, we have developed the computational approach to build a multi-

parameter model to quantitatively estimate the degree of methylation after parameterizing CpG content and other inherent sequence biases. This model is then used to predict the extent of methylation at each region of methylation identified by the MACS software across the entire genome. We have now validated this approach by comparing the DNA methylation estimates to other independent methodologies including the Infunium 450k Methylation array platform. The advantage of our qMBD-seq approach with these newly developed computational methods is that we can obtain genome-wide methylation data without bias to specific regions. With this method in hand, we are now ready to compare DNA methylation levels between African American and European-American prostate cancer tissues in a genome-wide level.

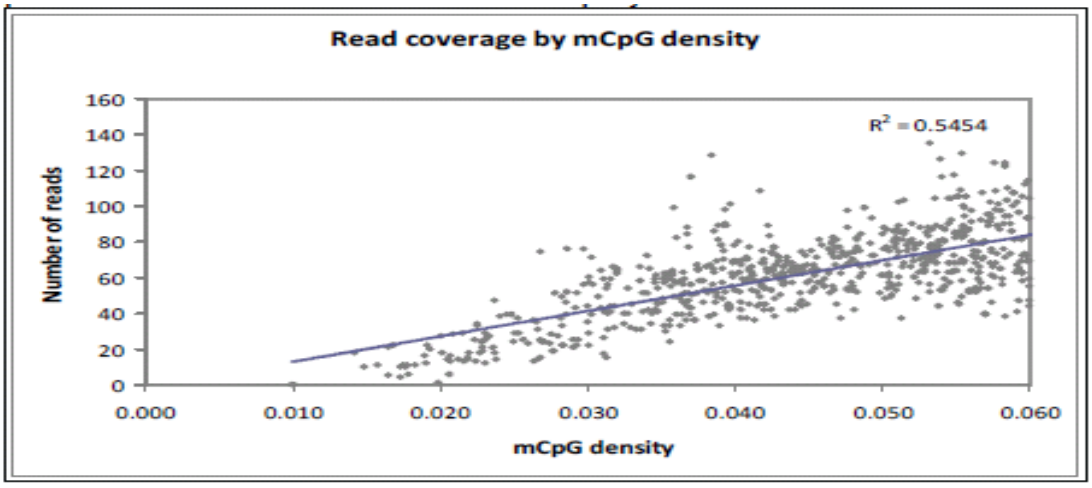

Figure 9. Number of reads in any 150 bp window is highly linearly related to the true density of actual methylated CpG (mCpG) in that window suggesting that our qMBD-seq assay is highly quantitative.

Task 3: Identify, process and isolate DNA from fresh frozen prostate tissues from EA and AA subjects as described I proposal (months 6 – 12).

We have now identified fresh frozen tissue blocks from several European-American and African-American prostate cancer subjects. Many of these have now been sectioned and subjected to DNA isolation. We expect to complete these activities in the next two months.

Task 4: Carry out genome-wide DNA methylation analyses on samples (months 12 – 24).

Data collection for these samples using the qMBD-seq approach is already under way. We expect to complete genome-wide analysis of DNA methylation of the European-American and African-American prostate cancer specimens in year two of the project.

In a parallel approach, we have examined the methylation status of Infunium 450K (484,968) CpG sites that corresponds to 21,221 genes in DNA methylation array (illumina) analysis. The 450K microarray includes CpG islands/shores/shelves/open sea, non-coding RNA and sites surrounding the transcription start sites for coding genes, but also for the corresponding gene bodies and the 3'-UTR. In all, we selected 25 promoter associated novel CpG sites that were differentially methylated in prostate cancer progression from benign to HGPIN to prostate cancer (FDR adjusted p-value <0.05; b value ≥0.2; fold change > 1.5). Pathway analysis of the genes with altered methylation patterns identifies the involvement of a cancer related network of genes whose activity may be regulated by *p*53, *Myc*, *TNF*, *IL*1&6, *IFN*-g and FOS in prostate pathogenesis.

Aim 2: Validation and quantification of genomic DNA methylation changes in prostate tissue samples from African American versus European-American men (months 0 – 30)

Task 1: DOD regulatory review and approval processes for studies involving human samples. Perfect numerical methods for analysis of genome-wide DNA methylation data. (0 – 6 months). As described above, these tasks have been completed.

As a preliminary analysis, we have validated several novel methylated genes identified from the illumina infunium 450K genome wide analysis in prostate cell lines derived from both African-American and European-American patients. The immortalized normal prostate epithelial cell line RWPE1, and the human prostate cancer cell lines, PC3, DU145 and LNCaP and benign prostatic hyperplasia (BPH) cell line were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The immortalized normal prostate epithelial cell line pNT1A was obtained from the European Collection of Cell Culture (Salisbury, U.K.). The above cell lines were all derived from European-American patient samples. The androgen-dependent prostate cancer cell lines, E00D6AA and androgen-independent cell line, MDA-PCa2b was derived from African-American prostate cancer patients and obtained from ATCC. All cell lines are maintained in RPMI supplemented with fetal bovine serum (10%) (FBS; Invitrogen, Carlsbad, CA) unless stated otherwise. The androgen-dependent prostate cancer cell lines HPC1L and HPC8L were derived from African-American prostate cancer patient at Howard University under Howard University Institutional Review Board approval. The HPC1L and HPC8L cell lines are positive for androgen receptor, prostate specific antigen and epithelial

membrane antigen. The HPC1L and HPC8L cells are maintained in RPMI supplemented with cholera toxin (2.5ng/ml); epidermal growth factor (10 ng/ml); insulin (5 ng/ml); hydrocortisone (100 pg/ml); phosphoethanolamine (0.005 mM); and selenious acid (45 nM). These cell lines were used to investigate the methylation frequencies as described in Task 4 below.

Task 2: Acquisition of prostate tissues from European-American and African-American Men. Obtain prostate tissues from both African-American and European-American men who have undergone radical prostatectomy (200 formalin fixed paraffin embedded (FFPE) samples from each racial group matched for age, PSA and Gleason Score) (months 6 – 24).

This task is now underway. Matched tissue blocks from 100 matched African American and European-American subjects (200 total) have been identified. A tissue microarray of these blocks is being constructed. Cores from each of these blocks in regions of cancer and normal are now being collected. We expect to complete these activities in year 2 of the project.

Task 3: Extraction of DNA from specimens obtained in task 3. (months 8 – 30).

DNA isolation from a subset of these specimens has already begun. We expect to complete these activities in years 2-3 of the project.

Task 4: Screening and validation of 20 differentially methylated genes and 5 that have been previously identified through preliminary studies (months 12 - 30).

This task is now under way for genes previously known to show cancer specific DNA hypermethylation in prostate cancer. As novel markers are identified through the ongoing genome-wide efforts, we will validate these new markers in the large cohort of AA and EA prostate cancer specimens during years 2-3 of the project.

Task 5: Explore the CpG island of 5' upstream regions based on their mRNA sequence by using Human BLAT Search engine (http://www.genome.ucsc.edu/cgi-bin/hgBlat) (months 12-13). These activities are now under way. They will be carried out for each novel biomarker that is discovered and prioritized through the genome-wide efforts.

Task 6: Design bisulfite primers based on bisulfite-converted sequence from the CpG island. For each gene, optimize pyrosequencing reaction by designing 2 or 3 pyrosequencing assays (months 14-16)

These activities are now underway. They will be carried out for each novel biomarker that is discovered and prioritized through the genome-wide efforts.

Task 7: Perform large scale and high throughput pyrosequencing reaction for each methylated gene using modified DNA from prostate tissues and analyze data (months 6-30). The methylation status of 25 novel methylated genes identified from the Illumina Infunium 450 K genome-wide array were validated in a panel of 10 prostate cell lines derived from both African-American and European-American patients as described in task 2 above. We explored the CpG island using the Human BLAT search engine and

designed bisulfite primers based on bisulfite-converted sequence from the CpG island. Table 1 shows the 25 novel genes identified and their function.

Gene Abbreviation	Gene Name	Locus	function
EPHA5	Ephrin type A receptor 5	4q13.1	Receptor tyrosine kinase
EPDR1	Ependymin related protein 1	7p14.1	Neuronal Regeneration
NOS1	Nitric oxide (NO) synthase 1	12q24.22	Produces NO
PLXNC1	Plexin C1	12q23.2	Viral receptor
CHRNB1	Cholinergic receptor, nicotinic beta 1	17p13.1	Nicotinic receptor
BNC1	Basonuclin 1	15q25.2	Transcription Factor
FZD1	Frizzled family receptor 1	7q21.13	Wnt signaling
RPL39L	Ribosomal protein L39-like protein	3q27.3	Related to Ribosome
SYN2	Synapsin 2	3p25.2	Neurotransmitter regulation
LMX1B	LIM homeobox transcription factor 1, beta	9q33.3	Transcription Factor
CELF2	CUG triplet repeat RNA-binding protein	10p13	Post-transcriptional regulation
SHC4	Src homology 2 domain containing family member 4	15q21.1-q21.2	Ras signaling
MYO5C	Myosin VC	15q21.2	Transferrin Trafficking
JAK2	Janus kinase 2	9p24.1	Cytokine receptor signaling
ZFP91	Zinc Finger Protein 91	11q12.1	Ubiquitination
PURG	Purine rich element binding protein G	8p11	Transcription initiation
SLC25124	Solute carrier family 25 member 24	1p13.3	Calcium-dependent mitochondrial solute carrier
PAQR5	Progestin and adipoQ receptor family member v	15q22.31	Progesterone signaling
LEO1	Component of Paf1/RNA polymerase II comples	15q21,2	RNA processing
PCSK9	Proprotein convertase subtilism type 9	1p32.3	Cholesterol homeostasis
CYB5R2	Cytochrome b5 reductase	11p15.4	Drug metabolism, cholesterol biosynthesis
ALDH1L1	Aldehyde dehydrogenase 1 family member L1	3q21.3	De novo purine biosynthesis etc
SERPINB9	Serpin peptidase inhibitor, clade B member 9	6p25.2	Granzyme B inhibitor
CXXC5	CXXC-type zinc finger protein	5q31.2	NF-kappa B/MAPK pathway
ZNF783	Zinc finger family member 783	7q36.1	Transcriptional regulation

Table 1.

Table 2 below shows the bisulfite primer designed based on bisulfite-converted sequence from the CpG island for most of the novel genes identified in Table 1. For some genes we tried commercially available pyrosequencing primers but reverted to designing our own primer sequences that were more cost effective.

No.	Pyrosequencing	Sense	Antisense	Sequencing primer
1	ADARB2	AGTTATATGGTGTATATATTTTGGATTAAG	Biotin-AAAACCTCTTCCTAAAAACAAACC	TTGGGAATTGGTATGGA
2	ALDH1L1	AGAGGGAGGTAGGATAGTAGATTTTATA	Biotin-AATTCTTTCCTCTCCTAAACAAATAC	GGTTTAGTTATAGAGAGTTTAGGTT
3	ALDH5A1	GGGAGTAATTTTAGGAGAATGTTTAGATA	Biotin-ACCTACCCTAAATACCAAAACC	AAAAAGTAGTTAGGTAGTAGA
4	BNC1	TGAAGGTATTTGTTGGTAAAAGATAT	Biotin-AAAAAAAAAAACCCAATCATCTCCTAAA	GGTATTTGTTGGTAAAAGATATAG
5	CXXC5	TGTGTGTGTATGGTGTATGT	Biotin-CTCAAAAAACCTAAATCACCCCATCCC	AAGTGTGTGTTTGGG
6	CYB5R2	GGTTTTGGGTTAGTTTTGTTTTTAGG	Biotin-ACCTTCTCCAACCTTACC	GGTTAGTTTTGTTTTTAGGG
7	FZD	GGGAAGTTTTAGTATTGTTTTTAAAGATG	Biotin-CCTCTCCTCCCAAAATCTCT	ATTTTATTTTGTTTTTTGGAATTA
8	GFPT2	AGATTTTAGTTGAGGGTTTAGGA	Biotin-AACCCTAACCCAAATCTATACAAACCT	GGATGTTTGTTTAGTTTGG
9	GNG7	ATGAGGTTTGTTTTTTTTTTAAAAGTTAAT	Biotin-CCCTAAACTCCTACTTTTTTCC	AAAGTTAATAGTTGGTGAAT
10	HES2	GGATAGTAGAGGGAGGGATTTGTATTTTTA	Biotin-ACTAAAAACCCTTATCCAAACT	GGATTTGTATTTTTAGTTGTTTTAT
11	HES5	GGTTTGGAGTTTGGAGTT	Biotin-ACCCACCTACTCCTTAAA	CCCCCCCCAATTCACAAACAATTTA
12	ITPKA	AATGTTGGAGGTGGATTTTGAAGTT	Biotin-CCTACCTCACCTTAATACCC	AGTTTTTAAGGAGGAGGAGT
13	LMX1B	GGGATTGATTAGAAGAGAGAGGT	Biotin-AAATAACCAACCCTAAAAACCTAC	GTTGGATTTTTGGGATTAA
14	MAFB	GGTTTAGGGTTATAGGGTGAGAAATTTT	Biotin-CCCAAACCCAATCTAAATACCT	GGTTATAGGGTGAGAAATTTTT
15	OPT	TGGGGTTAGTATAGATTTTAGGGTATAG	Biotin-CATATTAACCCCCCAACCCAAAAC	ATTTTAGGAGTAGGTTTATTGAGT
16	PTPRF	GTTTTTGGGTATTTTTGAAGGTTTTTG	Biotin-AAATCCTCCCACTAAACTTTCC	TTTATTAGGTTATGGTTTTGAGA
17	RPL39L	AGTAGGTTTGAGTTGTGGTATTG	Biotin-ATAAATAAAAATTCCTCCTCACC	TGTGGTATTGTTGTTTTTAG
18	SERPINB9	AAGAGGTGTTTGTAGAGGTTTTAG	Biotin-CCCTCACCTACCACCTAAT	GGGGTAGGAGTTTAGGTT
19	SLC16A11	GTTGTTTAAGGGGTTTGAAAGGG	Biotin-AAAATAAAAAACCCACCTAATTACC	GGGGTTTGAAAGGGG
20	SLC17A6	GGGGGTAAGGTTATTAAGGAGGT	Biotin-CACCCAAACCTTCCTCTCAAT	GGTTATTAAGGAGGTGG
21	SYN2	AGGTTTAGTTGGGAGGAGGT	Biotin-ACTATTTCCTAAAAACCTTTCCTATTAC	GGAGGTGAGGGTTGAG
22	TRIM15	AGGTTTTTGTTTTAAGTTGAAAGGTTGAAG	Biotin-AACTCAAATCCCCTATTCTCT	AAAGGTTGAAGTGGG
23	ZNF783	ATGGGAAGGGATAGGTTTAG	Biotin-CTCTCTTATAAACCACACAATCTTTCTCT	TTTAGGGTTAAGTATTTATTAGAGG
	Table 2 Pyrosequencing primers			

To validate the quantitative methylation level as determined by the illumina methylation array, we used pyrosequencing analysis for the 25 novel genes in normal and prostate cancer cell lines derived from both African-American (AA) and European-American (EA) men. A heat map of hierarchical cluster analysis for the methylation status in the prostate cell lines in shown in figure below. The results demonstrated higher methylation frequency for majority of the genes analyzed in prostate cancer cell lines with compared with the primary immortalized normal prostate cells lines. Interestingly, we observed significantly higher methylation frequency in the prostate cancer cell lines that were derived from the AA when compared with EA prostate cancer cell lines. Overall, the

methylation data derived from pyrosequencing analysis collaborated with the illumina infunium array data.

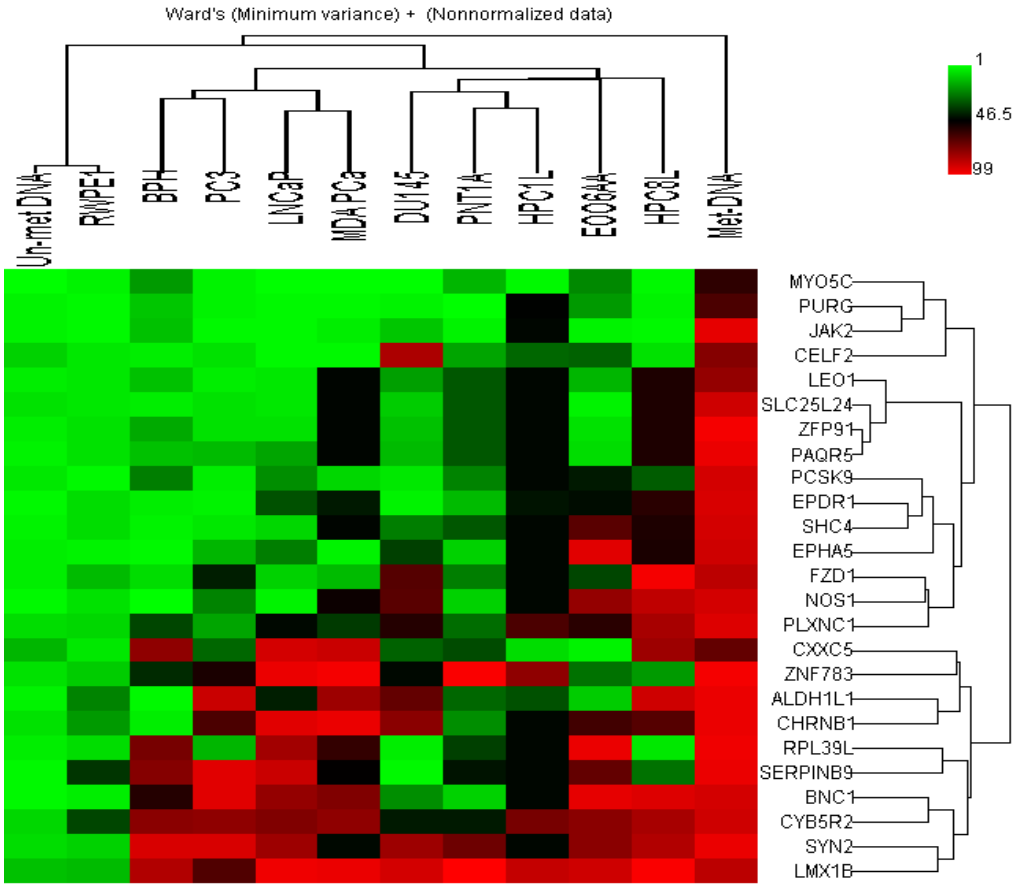

Figure shows hierarchical cluster analysis of gene validation of 25 novel selected promoter associated DNA methylated genes in a panel of prostate cell lines: RWPE1: Primary immortalized prostatic epithelial cells; PNT1A, primary immortalized epithelial cells; BPH; EA prostate cancer cell lines are LNCaP, PC3 and DU145; AA prostate cancer cell lines are MDA PCa2b, HPC1L, HPC8L and E006AA.

We will validate these new markers in the large cohort of AA and EA prostate cancer specimens during years 2-3 of the project.

KEY RESEARCH ACCOMPLISHMENTS:

- We have developed the qMBD-seq genome-wide DNA methylation technology as an approach that features the use of the methyl-binding domain of the MBD2 protein (MBD2-MBD) to bind and enrich methylated DNA fragments with high avidity and specificity. We have developed internal standards to obtain genome-wide methylation data without bias to specific regions. This approach will over-come some of the signal biases and challenges in quantitative determination of DNA methylation and assessment of differential methylation across sample groups.
- We have used the illumina infunium 450K genome-wide methylation array to identify 25 novel methylated genes that are differentially methylated in primary immortalized prostatic epithelial cell lines and prostate cancer cell lines derived from both African-American and European-American patients. Hierarchical cluster analysis shows higher methylation frequencies in the prostate cancer cell lines derived from the African-American patients in comparison with their European-American counterparts.

REPORTABLE OUTCOMES:

- AACR Annual Meeting: Abstract title "Genome wide methylation array of human prostate tissues using Illumina infunium 450K bead chip reveals distinct DNA methylation signatures with potential as clinical predictors" 2012. Chicago, IL.
- NCI Exploratory/Developmental Grants Program for Basic Cancer Research in Cancer Health Disparities (R21; PAR-12-094) entitled "Correlating DNA

Methylation Patterns with mRNA Expression of Prostate Tumors in African-American and European-American Patients for Biomarker Discovery" June 2012.

- Manuscript entitled "Identification of distinct DNA Methylation Signatures in the Human Prostate Cancer Tissues using Illumina Infunium 450K bead Chip Genome-Wide Methylation Array" is in preparation for submission to the *Epigenetics Journal*.

CONCLUSION:

Our preliminary data using the Infunium 450K methylation array platform has demonstrated proof of principle that the quantification of methylation status by genome-wide arrays can be collaborated by single-gene based analysis such as pyrosequencing.

Our on-going genome-wide methylation technology is the qMBD-Seq approach that features use of the methyl-binding domain of the MBD2 protein (MBD2-MBD) to bind and enrich methylated DNA fragments with high avidity and specificity. The advantage of our qMBD-sequencing approach over other genome-wide approaches is that we can obtain genome-wide methylation data without bias to specific regions, with this in mind we are now ready to compare DNA methylation levels between African-American and European-American prostate cancer tissues in a genome-wide level.

FUTURE WORK WILL FOCUS ON:

- Validation and quantification of genomic DNA methylation changes in prostate tissue samples from African-American versus European-American men (0-30 months): One of the specific aims of the project is to carry out validation and

quantification of genomic DNA methylation changes in prostate tissue samples from African American versus Caucasian men. To accomplish this, we will obtain prostate tissue samples from both African American and Caucasian men who have undergone radical prostatectomy (200 formalin fixed paraffin embedded (FFPE) samples from each racial group matched for age, PSA and Gleason Score). As described above, this task is already underway. Briefly, genomic DNA samples will be extracted from these specimens. Screening and validation of novel genes is already underway in prostate cell lines derived from both African-American and European-American patients. Already 25 novel genes have been screened in these cell lines. As novel genes are identified through the on-going genome-wide efforts, we will validate these new markers together with the already identified novel genes in the large cohort of AA and EA prostate cancer specimens.

Comprehensive evaluation of the biological function of novel genes differentially methylated in prostate tissue samples from African American versus Caucasian men (months 24-36). We will carry out gain-or-loss of functional studies using forced expression or gene knockdown (by siRNA/shRNA) of novel gene(s) with potential regulatory function in prostate cancer. To accomplish this, we will **o**btain the full length cDNA EST clone from Image consortium or by PCR amplification and clone it the cDNA into a suitable vector such as pCEP4 or pcDNA (Invitrogen) or purchase recombinant plasmid expressing gene of interest where commercially available. We will use the recombinant vector or siRNA/shRNA in transient and stable transfections where appropriate of pNT1A,

DU145, PC3 and LNCaP. We will confirm over-expression or down-regulation at the mRNA level by quantitative RT-PCR analysis and confirm protein expression of transfected cells by Western blot analysis by gene-specific antibody or to a vector-specific tag. Transfected cell number will then be determined by direct counting with a Coulter counter each day over a 6 day period. If novel gene is a negative regulator of prostate cancer, then induced gene expression should show decreased prostate cancer cell proliferation. It is also possible that inappropriate gene expression will induce apoptosis by markedly interfering with cell growth, particularly after several days. We will therefore directly determine apoptosis (TUNEL), proliferation (BrDU incorporation) as well as cell number and correlate these findings. For validated genes if antibody reagents useful for immunohistochemistry exist, we will consider carrying out immunostaining to assess gene expression in prostate tissue samples and correlate with prostate cancer clinicopathological data (months 30-36).

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Presentation Time: Wednesday, Apr 04, 2012, 8:00 AM -12:00 PM

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Poster Section:

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Prostate cancer (PCa) harbors a myriad of aberrant genomic and epigenetic alterations. Epigenetic inactivation of genes in PCa is largely based on transcriptional silencing by aberrant CpG methylation of CpG rich promoter regions. Candidate gene-based studies have identified a handful of aberrant CpG DNA methylation events in PCa. To better understand the role of aberrant epigenetic alterations and identify biological pathways likely to be affected by methylation mediated alterations in gene expression in PCa, we have examined the methylation status of 450,000 (450K) cytosine microarray (illumina). The 450K microarray includes CpG islands/shores/shelves/open sea, non-coding RNA and sites surrounding the transcription start sites for coding genes, but also for the corresponding gene bodies and the 3'-UTR. We demonstrate that the 450k DNA methylation array can significantly detect CpG methylation changes in radical prostatectomy prostate tissue samples. Cluster analysis revealed distinct DNA methylation profiles for prostate cancer tissues in comparison with non-malignant tissues. A total of 225 CpG loci ($p > 0.00049$) showed differential methylation in the cancer tissues in comparison with non-malignant tissues. Each of the 225 CpG locus was validated in silico using an independent, publicly available methylome dataset from the Cancer Genome Atlas. The majority of these loci were informative particularly in the prostate cancer. Overall, cluster analysis showed gene set enriched in pathways including transcriptional modifications, apoptosis/autophagy, chromatin assembly, growth factor signaling, cell cycle regulation and oxidative-redox reaction. This study shows that CpG locus methylation arrays could reveal important biological differences in the epigenome between prostate cancer and non-malignant tissues and provide potential markers for disease detection and/or progression.